Gut fermentability of supramolecular assemblies: A case study on ?-lactoglobulin and ovalbumin nanoparticles and amyloid-like fibrils

REFAEL G. (1), ROMANO A. (1), ENGELBERG Y. (2), TURJEMAN S. (3), KOREN O. (3), LANDAU M. (2), LESMES U. (1)
1 Department of Biotechnology and Food Engineering, Technion Israel Institute of Technology, Haifa, Israel
2 Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel
3 Azrieli Faculty of Medicine, Bar Ilan University, Safed, Israel

Processing may induce protein structuring, for example amyloid-like protein fibrils may form during spray or freeze drying. However, the link of amyloids in human pathogeneses raises concern over their formation in foods. Therefore, this study fabricated various protein architectures, characterized them, and studied their digestive fate.
Changes in pH and temperature (pH=2, 37-80°C) were applied to ?-lactoglobulin (BLG) or ovalbumin (OVA) to fabricate amyloids fibrils (AF) that were characterized by DLS and TEM as well as Thioflavin T assay to affirm amyloid arrangement. These assemblies were subjected to semi-dynamic *in vitro* digestion coupled to LC-MS/MS proteomic analyses and compared to the digestion of the native protein counterparts. In respect to microbial fermentability, amyloid structures were screened for antimicrobial activity against gram negative and positive bacteria, as well as tested for their colonic fermentability using *in vitro* anaerobic fermentations of freshly collected human feces (n=5). Microbiota responses were studied using 16S sequencing and QIIME2.
This work provides evidence that amyloid structures attenuate the digestive proteolysis of BLG and OVA in the upper GI. This is shown by reduction in the abundance of bioaccessible peptides upon digestion. Antimicrobial activity of OVA (e.g., MIC=21mg/mL against *micrococcus luteus*) was abolished by amyloid formation. Colonic fermentation results strengthen this finding as alpha-diversity scores (observed features) were significantly lower ($p<0.003$) for OVA (121±29, n=13) in comparison to OVA-AF (161±27, n=17). This reduction in biodiversity was specific for OVA, as BLC (166±21, n=12) and BLC-AF (166±27, n=16) were similar to FOS (160±30, n=15). Beta-diversity analysis shows OVA fermentation shifts the ecosystem compared to OVA-AF ($p<0.05$) and ANCOM-BC analysis shows specific symbionts of the gut are significantly reduced in relative abundance in the presence of OVA (e.g., *Roseburia*).
Overall, structuring proteins into amyloids attenuates their proteolysis in the upper GI with mixed implications on the diversity of the colonic microbiota. Thus, this work shows that processing can be harnessed to fabricate novel functional protein architectures; however, their digestive fate requires further study to support or refute possible benefits or deleterious effects. Altogether, this research provides evidence that show amyloid structures may be engineered to modulate gut nutrition.